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Evaluation of bitter leaf (*Vernonia amygdalina*) extract in the inhibition of fungi causing post harvest rot of tomato fruits in Makurdi, Benue State, Nigeria

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ABSTRACT

The study was carried out to evaluate bitter leaf (Vernoniaamygdalina) extract in the inhibition of fungi causing post-harvest rot of tomato fruits in Makurdi, Benue State. Nigeria. Tomatoes have been in cultivation in Nigeria for a very long time, major producing areas lies between latitude 7.5^{0}N and 13^{0}N and within temperature range of 25^{0}C- 34^{0}C. The method employed was to isolate and identify the pathogens causing rot from tomatoes. The fungal pathogens isolated were Fusarium oxysporum and Aspergillus niger.The fungi were cultured with the bitter leaf extracts using potato dextrose agar (PDA), and mycelia growth and zone of inhibition was observed. The study revealed that there was significant differences (P=0.05) in the Grand mean inhibitory effect in both test fungi with the control with increase in concentration. At 75g/ml concentration, plant extract hasthe highest inhibitory mean effect on Aspergillus niger (0.62±0.06mm) and Fusarium oxysporum(0.3±0.09mm). V. amygdalina have inhibitory potential on post harvest rot causing fungi of tomato in storage and suggest its ability to prolong its shelf-life.

Key words: fungal pathogens, plant extracts, tomatoes, Makurdi

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INTRODUCTION

Tomato (*Solanum lycopersicum*) is one of the most important vegetable crops in the world. It is the world's largest vegetable crop after potato. Tomato has been in cultivation in Nigeria for a very long time, major producing area lies between latitude 7.5^{0}N and 13^{0}N and within a temperature range of 25^{0}C -34^{0}C [1]

Tomato is an important condiment in daily diets, consumed both fresh and in paste form and a very cheap source of vitamins A,C,E and minerals which protect the body against diseases. It contains lycopene, a flavonoid antioxidant together with carotenoids which protect the body cells and other structures in the human body [2].

Nigeria ranks the 16thlargest tomato producing nation in the world;the production of tomatoes in Nigeria in 2010 was about 1.8 million metric tonnes which accounts for about 68.4% of West Africa, 10.8% of Africa's total output and of the world output [3]. However, the demand for tomato and it's by products still outweighs the supply.

Despite the importance of the tomato fruit, it is highly vulnerable to a cocktail of fungi and bacteria pathogens leading to post harvest losses. The post-harvest diseases of tomato fruit cause considerable losses during storage and transportation. These diseases are managed principally by application of synthetic fungicides, however

increasing concern for health hazards and environmental pollution and cost effectiveness of chemicals has required the development of alternative strategies of the control of post-harvest tomato diseases.

Leaves of bitterleaf plant possess some anti-microbial properties against a wide range of pathogenic fungi [4]. They are also used for culinary, medicinal and curative purpose.

Therefore this research was carried out to evaluate the effect of bitter leaf extract on fungi causing post harvest rot of tomato in Makurdi.

MATERIALS AND METHODS

Collection of samples
Tomatoes showing symptoms of rot were collected in polyethylene envelopes from major markets in Makurdi and taken to the laboratory for isolation of fungal pathogens.

Preparation of culture medium
Potato Dextrose Agar (PDA) was used for isolating fungi and it was prepared according to manufacturers' instruction.

Isolation of fungal Pathogens
Small pieces of the rotten part of the tomato samples were picked using a sterile scalpel. These were surface sterilised in 0.1% sodium hypochlorite for 30 seconds and then rinsed in sterile distilled water for 30 seconds. The pieces were dried on sterile filter papers and transferred on to fresh solidified Potato Dextrose Agar (PDA) medium impregnated with streptomycin and incubated for 7 days at 28 - 30^0C. Three replicates were made for each sample. After 7 days of incubation, isolated colonies were picked with sterile wire loop, streaked into solidified PDA and incubated for 5 days. Isolated colonies were re-inoculated into fresh petri dishes and incubated until pure cultures were obtained.

Identification of fungi
Visual observation in Petri dishes and micro morphological studies in slide culture were done for identification of the fungal isolates. Colony characteristics such as appearance, change in medium colour and growth rate were observed. Shape of the conidia and conidiophore were taken note of. These features were matched with standards in Barnett and Barry(1972).

Pathogenicity test
Fresh tomato fruits were rinsed in sterile distilled water and allowed to dry after which pure cultures of the isolates were inoculated into the fruits using a sterile wire loop. Solidified PDA was used to seal each inoculum in the wound to prevent contamination by other micro-organisms. Controls were tomato fruits inoculated with sterile PDA. These were arranged on laboratory desks in complete randomized design. Re-isolation and re-examination of fungi was carried out according to Koch's Postulates.

Collection of plant material
Fresh leaves of the bitter leaf plant (*Vernoniaamygdalina) were* collected in polyethylene bags and transported to the botany laboratory of the Benue state University for preparation of the extract.

Preparation of extract
50 grammes of fresh matured leaves of the bitterleaf plant were thoroughly rinsed in running tap water and air-dried in the laboratory. These were ground with pestle in a mortar to facilitate extraction. The macerate was dissolved in 100ml of distilled water and then strained through a 45 micro-mesh. The filtrate was dispensed into universal specimen bottle.

Concentration of crude extracts
Serial dilutions of the crude extract were prepared to give different concentrations of 25g/ml, 50g/ml and 75g/ml.

Susceptibility Test

2mls of the extract concentration was dispensed in Petridishes after which 15-20mls of molten PDA was added. The Agar-extract mixture was swirled gently on the work bench to ensure even dispersion of the extracts and allowed to solidify.4mm diameter of mycelia obtained from the edge of a five day old culture of each test fungi was inoculated centrally into the medium. Four replicates were used for each fungal isolate. Controls were Petri plates with the organism with no botanical extract. The Petridishes were arranged in complete randomized design & incubated at 27-30 ^{0}C for 5-7 days. Inhibition for fungal growth was calculated using the formula:

$$\frac{L1 - L2}{L1} \; x \; 100$$

Where L_1-growth of the pathogen in control
L_2-growth of the pathogen with treatment

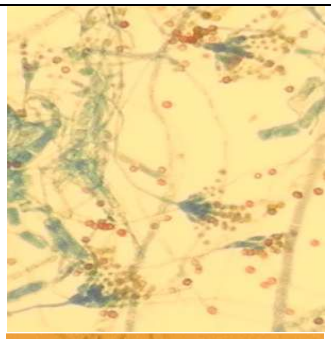
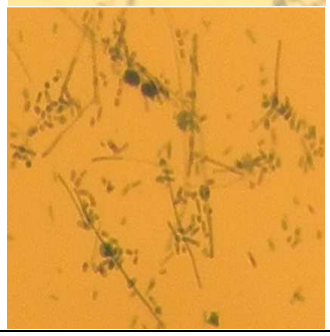
Data Analysis

Data obtained from the study was analyzed using Analysis of variance (ANOVA) and the Fishers least significant difference was used to separate the means at 5% level of significance.

RESULTS

Table 1 indicates the culture and morphological characteristics of the identified fungi, *Aspergillus niger* and *Fusarium oxysporum.* And they were found to be pathogenic using Koch's postulate.

Table 1: Characterization of Fungal isolates from Tomato fruits

Macro/Microscopic Characteristics	Appearance on PDA	Photomicrograph	Probable Organism
Colony bears abundant and erect and usually crowed conidial structures, carbon black but sometimes deep brown black. Conidia heads are split into two or more loose to reasonable well defined columns. Conidiophores are smooth and hyaline.			*Aspergillus niger*
Colonies are rapid in growth. The mycelium is usually white then becoming orange. Microconidia are abundant and have a characteristic bean shape. Chlamydospores intercalary			*Fusarium oxysporum*

Effects of Pathogenicity test of fungi inoculated into healthy tomato

The Table below shows the result of pathogenicity test of the fungi inoculated into the healthy tomato fruits had the same features as the ones re-isolated from them, indicating that the fungi were responsible for the spoilage of tomato fruits. *Aspergillus niger* produced the higher rot in the tomato fruits, with a rot diameter of 1.14cm while *Fusarium oxysporum* produced the lower rot diameter of 0.18cm in the tomato fruits.

Table 2: Effects of pathogenicity test of fungi inoculated into healthy tomato

Treatment	*Aspergillus niger*	*Fusarium oxysporum*
Control	0.00±0.00[a]	0.00±0.00[a]
R1	5.00±0.00[b]	5.00±0.00[b]
R2	4.50±0.50[b]	4.50±0.50[b]
R3	5.00±0.00[b]	5.00±0.00[b]
R4	4.50±0.00[b]	4.00±0.00[b]

Footnote: means tagged with different letter alphabet is significant at P= 0.05
Key: Mean±SEM

Percentage Inhibition of Plant Extracts on *Fusarium oxysporum*

The Table below shows that there is significant difference between the level of concentration (25g/ml, 50g/ml and 75g/ml) in the inhibition of the bitterleaf extract and control observed on the media. The result showed that increase in concentration is significantly proportional to increase in zone of inhibition (P <0.05). The mean effect of difference between of 0.1cm at 25g/ml and 4.67cm for control was significantly different. The concentration 25g/ml of the extract showed a considerably inhibitory effect on *Fusarium oxysporum.*

Table 3: Percentage inhibition of plant extracts on *Fusarium oxysporum*

Concentration	*Fusarium oxysporum*
25g/ml	0.1±0.44[a]
50g/ml	0.2±0.08[a]
75g/ml	0.3±0.09[a]
Control	4.67±0.96[b]
FSLD (0.05)	1.50

Footnote: means tagged with different letter alphabet is significant at P=0.05
Key: Mean ± SEM

The effect of Percentage inhibition of bitterleaf plant extract on *Aspergillus niger*

Table 4 below shows that there was inhibitory effect of aqueous extract of bitterleaf on the *A.niger*. The fungus showed the highest inhibitory effect at 75g/ml (0.62±0.06cm) and the least inhibitory effect at 50g/ml (0.76±0.01cm).

Table 4: Percentage inhibition of bitterleaf plant extract on *Aspergillus niger*

Concentration	*Aspergillus niger*
25g/ml	0.72±0.302[a]
50g/ml	0.76±0.01[a]
75g/ml	0.62±0.06[a]
Control	5.38±1.03[b]
FLSD (0.05)	1.60

Footnote: means tagged with different letter alphabet is significant at P= 0.05
Key: mean ± SEM

DISCUSSION

The fungi associated with the spoilage of post-harvest deterioration of tomato sold in major markets in Makurdi, Nigeria revealed the presence of *Aspergillus niger* and *Fusariumoxysporum*. These pathogens were consistently associated with post-harvest rot of tomatoes during storage. These pathogens have been previously linked with post-harvest tomato rot [5-7]. The pathogenicity test which confirmed that the pathogenic fungi inoculated on tomato fruit cause rot was due to the ability of the pathogens to utilise nutrients of tomato as substrate for growth and development. This is in consonance with reports on fungi associated with tomato fruits [8-9].

Generally spoiling fungi are considered pathogenic which could cause infections and allergies [10]. *Aspergillus spp* are known to produce several toxic metabolites, mycotoxin which is very important toxin worldwide because of the hazard it poses to human and animal health. [11-12] thus , extra care should be taken during handling, packaging, storage and transportation as these may eventually result in decay and increase in growth of micro-organism which become activated because of changing physiological state of the produce [13]. It has been pointed out that the inocula responsible for storage diseases among tomato often originates from infected and infested farm tools [14]

It was observed that the effect of bitter leaf extract prove to havefungitoxic effect to inhibit the rot pathogens, this suggests that the plant contain active compounds/ phytochemical that affect the growth of the rot pathogen. This agrees with the report of John et al, 2016[15] who stated that *V. amygdalina* was effective against the mycelial growth of *Fusarium oxysporum* (32.26%), *Rhizopus stolonifera* (22.58%) and *Geotrichumcandidum* (45.16%). *Vernoniaamygdalina* may have acted by the production of more antibiotics substances that inhibited the growth of *A.niger* and *F. oxysporum*. This has been reported by Onyeani et al., 2012[16].

There was similar trend in the fungitoxic effect of the plant extract with respect to concentration. The main effect of the levels of concentration (25g/ml, 50g/ml, 75g/ml) and the controls were significantly different. ($P< 0.05$) for *Aspergillus niger*. This is in consonance with observations of Anukwuorji et al (2012) [17] and that of Okigbo et al., 2009b, [18] all reported a significant ($P<0.05$) difference in the inhibitory effect of all the plant extract concentration.

CONCLUSION

The result of this study proved that *V. amygdalina* have inhibitory potential on rot causing fungi of tomato in storage and suggest its ability to prolong its shelf life. Prolonging the shelf life of the fruit would go a long way in reducing the scarcity of the fruit. It can be deducted from the study that aqueous extract of *V. amygdalina* could be an alternative to synthetic fungicides in controlling tomato rot. Aqueous extract is environmentally friendly, non-toxic, readily available and affordable. However, further research should be done to exhibit its amazing fungicidal properties that support their traditional use as antiseptics.

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